7.(Amended) A [drug ~~containing~~] pharmaceutical composition comprising a protein according to anyone of claims 1 to 6.

Please add the following claim:

--27. A protein according ~~to claim~~ 6 wherein said blocking group has a ~~mo~~lecular mass of approximately 43 atomic mass units.--

REMARKS

A Rule 62 continuation was filed in the above-captioned case on July 28, 1992. Claims 1-26 are pending. Applicants request that the preceding amendments and following comments be made part of the record before examination of the above-captioned application.

In the most recent office action, dated January 28, 1992, the examiner required restriction of the invention pursuant to 35 U.S.C. §121. Claims 1-7 ("group I"), drawn to proteins, and claims 8-26 ("group II"), drawn to DNA vectors and methods of producing proteins, were deemed to constitute patentably distinct species. At that time, provisional election was made, with traverse, to prosecute group I. Applicants now cancel claims 8-26, corresponding to group II, without prejudice to their right to file corresponding claims in a divisional application.

In the parent case, U. S. Ser. No. 07/659,408, claims 1-7 were rejected under 35 U.S.C. §112, second paragraph, for an alleged failure to particularly point out and distinctly claim the subject matter regarded by applicants as their invention. Specifically, the term "units" was said not to be defined, thus allegedly rendering all claims indefinite. In response, applicants direct the examiner to page 48, lines 14-24 of the present specification. The calculations for determining enzyme

units are set forth in detail therein with further reference to Bradford, M. M., *Anal. Biochem.* 72:248-54 (1976) (copy to follow). Applicants submit that the above-noted section of the specification renders use of the term "units" clear and definite.

The original specification also was objected to under 35 U.S.C. §112, first paragraph, for allegedly failing to provide an "enabling" disclosure. In particular, the specification was deemed inadequate because (1) "all proteins having substantial homology to the urate oxidase of the first claim are not enabled" and (2) all proteins that migrate at 33.5 Kda in all bidirectional gels are not enabled.

Regarding the first objection, the examiner is directed to the publication by Legoux *et al.*, *J. Biol. Chem.* 267:8565-70 (1992), a copy of which accompanies this paper. The Legoux report describes the cloning of the urate oxidase (UO) enzyme gene of *A. flavus*. This was accomplished by (1) amino acid sequencing of peptides derived from hydrolysis of purified UO and (2) generating oligonucleotide probes based on the amino acid sequence. The report also describes the full DNA-coding and protein sequences of *A. flavus* UO. The results show that this particular enzyme contains many of the conserved sequences found in UO's of other organisms. In other areas where the amino acid sequence itself is not preserved, the differences are, nonetheless, conservative of the chemical nature of the amino acids involved.

The success of the foregoing approach is practical evidence that the invention is, in fact, enabled, with respect to proteins "having a substantial degree of homology" with the claimed sequence. Once a DNA sequence is known, it does not require undue experimentation to find similar DNA sequences, and hence, homologous proteins. This can be done in the same manner as described in by Legoux *et al.*, where oligonucleotides were

designed to probe genomic libraries for unknown UO sequences. By adjusting the length of the probes and hybridization conditions, the degree of homology which one can detect may also be adjusted. This procedure is made easier when, as was the case here, other homologous DNA sequences are known. Identifying conserved sequences of the UO gene by comparison of *A. flavus* UO with baboon, pig, mouse, rat, human, *Drosophila*, and soybean UO, it was possible to target these regions with probes and thus maximize the chances of finding homologs.

The second objection to the specification involves the scope of claim 3, which reads

> A protein according to any one of claims 1 or 2, which presents, by analysis on a bidimensional gel, a spot of molecular mass of about 33.5 kDa and an isoelectric point around 8.0, representing at least 90% of the protein mass.

The examiner points out that a number of different bidirectional gel systems are available and suggests that applicants' description of a protein which migrates at 33.5 kDa on one such gel system will not enable proteins migrating at 33.5 kDa on all such gel systems. The first paragraph of §112 states:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms **as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same** and shall set forth the best mode contemplated by the inventor of carrying out his invention. (emphasis added)

Reading claim 3 in light of the specification, particularly Example 4, pages 22 (line 3) to 25 (line 13), one skilled in the art can, in fact, "make and use" the present invention. Applicants have, however, amended claim 3 in order to more

clearly define the bidimensional gel system being employed. Therefore, the objection is deemed obviated.

Claim 6 was rejected under 35 U.S.C. §112, second paragraph, with the term "preferably" deemed to render the claim indefinite. Without conceding this point, applicants have amended the claim to remove the objectionable term; the molecular mass preference now is highlighted in new, dependent claim 27.

Claim 7 stands rejected under 35 U.S.C. §112, second paragraph, based on the examiner's contention that the phrase "a drug containing" is confusing. We have implemented the examiner's suggestion and substituted the above-quoted language with "a pharmaceutical composition comprising."

Claim 1 stands rejected under 35 U.S.C. §102(b) over Laboureur *et al.*, U.S. Pat. No. 3,810,820. The Labourer patent describes the process for the manufacture of UO having "high activity." In fact, such high activity, when measured in terms of specific activity as defined in Example 9 of the present specification, page 48, lines 14-24, is about 8U/mg, approximately two times (claim 1) to four times (claim 2) lower than the specific activity recited in the present claims. As a result of an innovative purification regimen (see specification at page 15, line 4, to page 17, line 16), validated through extensive experimentation described in the accompanying declaration, applicants' claimed UO is of a greatly increased purity and thus exhibits a higher activity than the UO of the Laboureur patent. Since the Laboureur disclosure thus does not place in the hands of those skilled in the art a UO enzyme as presently claimed, Laboureur fails as a § 102(b) teaching. Withdrawl of the present rejection is therefore requested.

Claims 1-7 stand rejected under 35 U.S.C. §103 over (1) Laboureur *et al.* and (2) Laboureur *et al.* in view of Reedy *et*

al. and Riggs or Nielsen *et al.* Both of these rejections rely on the examiner's argument that the Laboureur patent makes obvious the further purification of UO from *A. flavus*.

The following is a quotation from the Laboureur patent, column 5, lines 38-55:

> It may be necessary to purify the substance still further, for example by means of a series of precipitations from aqueous media, generally fractional precipitations, using organic liquids miscible with water or aqueous solutions containing ammonium sulphate. It is also possible to make use of adsorption upon hydroxyapatite, bentonite and alumina and subsequent extraction, followed by elution using saline solutions. The purification can be carried still further by subjecting the thus treated products to chromatography, which may be a cyclic or non-cyclic process, by making use of columns of substances which make it possible to eliminate those impurities, in particular proteins, which are still present in the extract. The substances which can be used for this purpose include columns of cellulose ion exchange materials, dextrans and polyacrylamides. Elution may be effected by means of liquids in which there is a continuous or discontinuous change in the pH or in the molarity thereof.

But even if it is assumed, *arguendo*, that this passage from the Laboureur patent would have prompted one of ordinary skill to attempt a further purification, the fact remains that actual attempts thereafter to purify UO, using the standard protein purification procedures spelled out by Laboureur *et al.*, met with repeated failure (see discussion in the appended declaration). In short, the technology represented by the above-quoted section of the Laboureur patent was incapable of yielding a protein with the specific activity presently recited.

The accompanying declaration (original signed copy to follow) details the extensive experimentation that was required to purify UO to the level presently claimed. To summarize, after the Laboureur patent issued, affinity chromatography was developed. This approach employs a specific binding partner of

the protein to be purified to bind that protein to a column. The bound protein must then be eluted and recovered. Early attempts either failed to produce binding of the enzyme to the column or gave binding that could not be reversed without damage to the enzyme. Later attempts resulted in more favorable binding and elution but recovery remained a problem. Pseudo-affinity chromatography was attempted next. This approach employs protein-binding ligands to attach proteins to columns, similar to affinity chromatography. The ligand, however, is not a specific binding partner, thus hopefully making elution less difficult. Again, several unsuccessful attempts were made before an appropriate system was designed. After considerable experimentation, a five step process was developed. This process is set out in detail by the specification in Example 4 of the present application at pages 15, line 4, to page 17, line 16.

Based on the foregoing discussion and the attached declaration, the difficulty associated with purifying the UO claimed in the present invention is evident. It also shows why the Laboureur disclosure is inadequate to support a finding of obviousness with respect to the present application. Applicants therefore request that the present § 103 rejection be withdrawn.

In light of the preceding amendments and remarks, applicants believe the claims to be in proper form. Therefore, allowance of remaining claims 1-7 and 27 is respectfully requested.

Respectfully submitted,

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Date

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